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MATERIALS OF INTERNATIONAL SCIENTIFIC CONFERENCE "INTEGRATED CLINICAL AND PATHOGENETIC APPROACHES IN DIAGNOSIS AND THERAPY OF CANCER", JUNE 13–15, 2016, KYIV, UKRAINE

The International scientific conference "Integrated clinical and pathogenetic approaches in diagnosis and therapy of cancer" was held on June 13–15, 2016 at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine, Kyiv, Ukraine.

The focus of the conference was a search for the new fundamental, translational and clinical approaches in cancer research, prevention, diagnosis and treatment.

At the conference we have learned a lot of new data presented by researchers from many countries, namely Ukraine, Sweden, Latvia, Lithuania, Poland, and Belarus.

The Director of IEPOR, Academician Vasyl Chekhun gave an overview on the research that was and is performed at IEPOR, and also the future directions important to combat the cancer diseases. He discussed the concept of cancer stem cells, the importance of the multimeric interactions between cancer cells and normal cells, and microenvironment. Academician V. Chekhun highlighted that the research on stem cells were initiated at our Institute about half century ago by Academicians R. Kavetsky and Z. Butenko. And the First International Conference "Role of stem cell in leukemo- and carcinogenesis" summarizing the early developments in the field was held at our Institute at that time.

The new level of the cancer problem was presented by Professor Ingemar Ernberg from Karolinska Institutet (Sweden) who presented his recent concept of cancer cell "attractor", at the edge of tumor biology, mathematical modeling and system biology.

The keynote reports were presented also by Professor Ninel Berezhna, Doctor of Medical Sciences Victor Zhylchuk, Doctor of Sciences Iryna Kozeretska, Doctor of Sciences Olena Kashuba and Doctor of Sciences Denys Kolybo. The lecturers presented the up-to-date information on the role of connective tissue as the key factor of tumor microenvironment, the metastatic bone lesions and disseminated cancer cells in bone marrow, genetic aspects of cancer in Ukraine, the role of MRPS18-2 oncoprotein in regulation of cell differentiation, HB-EGF as potential oncomarker and target for anticancer therapy.

We have listen to many interesting reports, concerning the choice of the targets for cancer therapy, presented by Ukrainian researchers (see enclosed abstracts). Cancer therapy and the problems of personalized cancer treatment were also in the spotlight.

During conference, largely supported by the VACTRAIN of Horizon 2020 program, there were workshops with the training in wet lab on different modern research techniques, such as quantitative real-time PCR (qRT-PCR), Comet assay, Fluorescence activated cell sorting (FACS), and also immunohistochemistry. Many Master and PhD students and also young and experienced researchers took part in the workshops.

This conference was also a platform to popularize the European School of Oncology, represented by Dr. Wojciech Wysocki from Poland. We are sure that this information will increase mobility of our medical students.

During conference, we had a great opportunity to show our beloved country as it is to our guests. There were excursions around Kyiv and a boat trip on Dnipro, the travel to the regional center Chernihiv, the ancient city north from Kyiv.

We hope that all participants got a take-home message: we have a long and difficult way to go to cure cancer disease, and we hope we reach our goal in our life time.

PROGNOSTIC AND PREDICTIVE VALUE OF IODINE SYMPORTER IN HUMAN BREAST CANCER CELLS

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Breast cancer (BC) is one of the most serious problems due to the high incidence and increasing number of patients with tumors resistant to chemotherapy. In this respect, the metabolic iodine may have increasing relevance. In particular, there is an opinion, that iodine symporter can serve as a potential marker of tumor progression. We aimed to investigate the relationship between iodine symporter expression clinical and pathological characteristics of BC patients, and also to clarify its role in the determination of sensitivity to cytostatics. The 162 BC patients at stage II–III, undergoing hospital treatment in the Ivano-Frankivsk Regional Clinical Oncology Center during 2013–2015, were studied. All patients received neoadjuvant chemotherapy (NAHT), a course which included

a 2–6 scheme cycles FAC, AC intervals of 21 days. Efficiency of NAHT was evaluated after every 2 cycles, according to mammography and ultrasound diagnostic criteria. The largest number of tumors showing expression of iodine symporter was in the stage III patients (73.4%), low degree of differentiation (60.4%), and in three negative (basal) molecular subtype tumors (68.2%). There was a correlation between iodine symporter expression and a stage of disease ($r = 0.46$; $p < 0.05$); the presence of metastatic lesion in regional lymph nodes ($r = 0.49$; $p < 0.05$), the degree of differentiation ($r = -0.42$, $p < 0.05$), the basal molecular subtype ($r = 0.44$; $p < 0.05$), and tumor tissue sensitivity to NAHT (FAC, AC) ($p < 0.05$). ***Conclusions*:** High levels of iodine symporter in tumors indicate resistance to NAHT and may be used as an additional prognostic and predictive marker of BC.

HEPATITIS C VIRUS POSITIVE HEPATOCELLULAR CARCINOMA RECURRENCE FREE SURVIVAL AND THE UCKL-1 AND S18-2 EXPRESSION

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Despite recent advances in hepatocellular carcinoma (HCC) diagnosis and achievements in the treatment, a high risk of recurrence remains the Achilles heel of HCC care. Therefore, there is a need in reliable prognostic markers for proper follow up of patients with HCC. The uridine-cytidine kinase-like protein 1 (UCKL-1) involved in cellular nucleotide metabolism and mitochondrial ribosomal protein S18-2 as intracellular signaling regulator can be considered as putative candidates because of their importance in the tumor cells (including hepatocytes) transformation process. UCKL-1 and S18-2 expression has not been investigated yet in liver tissue and in HCC lesions. In presented study we explored UCKL-1 and S18-2 proteins value as hepatitis C virus (HCV) positive HCC relapse risk prognostic markers. 42 liver biopsies of HCV patients with successfully treated HCC were analyzed. HAI, fibrosis score, HCC differentiation and vascular invasion have been evaluated in every biopsy. Oncoproteins expression was visualized by immunohistochemistry staining with polyclonal rabbit anti-UCKL-1 and anti-S18-2 antibodies, using EnVision G2 double staining system. The average of expression was calculated by multiplying the frequency of stained cells and staining intensity. HCC recurrence in HCV cirrhosis was radiologically confirmed in 27 of the 42 patients. All patients were followed up from 1 to 8 years; HCC relapse appeared after 1–5 years. In relapses mortality rate was significantly higher than in the absence of HCC relapse: 59.3% *vs* 6.7%, respectively; $p = 0.001$. HCC relapse occurred more often in older patients (63.78 ± 9.22 *vs* 53.53 ± 4.07; $p < 0.001$). Patients gender, body mass, HAI and fibrosis score as well as portal hypertension signs, biochemical indices of liver damage, HCV genotype, and viral load did not influence HCC recurrence. In relapses HCC nodules were larger (50.44 ± 17.83 mm *vs* 41.47 ± 20.76; $p = 0.148$), vascular invasion was more common ($p = 0.02$), but HCC differentiation had no predictive value for recurrence. In relapses UCKL-1 staining was observed significantly more enhanced in the HCC nodules than in absence of recurrence (62.69 ± 50.4 *vs* 26.0 ± 30.19; $p = 0.006$). There were no difference in UCKL-1 staining in the unaffected liver tissue between relapsers and non-relapsers. Regardless of HCC relapse ($p = 0.378$) S18-2 staining was several times greater in the HCC nodules than in unaffected liver. HCC treatment method itself has also significant predictive value — in case of liver transplantation the recurrence rate was appreciably lower than after liver rejection or RFTT ($p = 0.001$). ***Conclusions***: It is likely that strong UCKL-1 signal in HCC nodules indicates the higher risk of HCC relapse after initial successful treatment, especially if it was not a liver transplantation. S18-2 signal is higher in the HCC nodules, than in the unaffected liver; however, it has no prognostic value.

ADVANCED LIVER RESECTION AND TUMOR PROGRESSION

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Despite the introduction into clinical practice a study on the functional reserve of the liver, calculating the volume of future residual stump of the liver, as well as the use of portal vein embolization or an associated division of liver and portal vein ligation — severe hepatic insufficiency (SHI) is the main cause of complications and mortality of patients. We aimed to find the new approaches to prevent SHI and to control SHI in the postoperative period. The developed scale and principles of SHI diagnosis described in (Nuh N. Rahbari et al., 2015) will be used in the future studies to standardize surgical complications in the postoperative period. The study of physiological changes in the tissues of the operated liver of cancer patients will improve diagnosis and control on SHI.

A REDOX STATE OF FATTY TISSUE IS A CRITICAL FACTOR IN THE PROGRESSION OF GASTROINTESTINAL TRACT TUMORS

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Studies on the involvement of mitochondria in metabolic changes in fat tissues (FT) adjacent to is important in understanding of the complex relationship between tumor and adipose tissue (AT) that contribute to the progression of cancer. Hypertrophied and dysfunctional AT at obesity is characterized by activation of the generation of superoxide radicals (SR), which is manifested by inflammation. We aimed to investigate the redox state of tumor adjacent AT and its relationship with metastasis of stomach (SC) and colorectal (CC) cancers. 11 samples of healthy individuals, 29 SC samples, and 27 CC samples of the patients at stages II–III (T2–4N0–2M0G2–3) were studied, using EPR (in the liquid nitrogen) and Technology Spin Traps, and also by immunohistochemistry, and statistics. We have found that the level of activity of the electron transport chain complex in mitochondria in AT adjacent to tumors correlated with the degree of differentiation of CC and SC ($r = 0.64$, $r = 0.47$; $p < 0.05$). Moreover, SR generation and and oxidation-induced DNA mutations is 3.5 folds higher, when compared to healthy individuals ($p < 0.05$). The high levels of SR generation in AT adjacent to tumors is associated with a high number of tumor-associated adipocytes, macrophages in tumor tissue, and with distant metastasizing ($p < 0.05$). ***Conclusions*:** Defects in the oxidative phosphorylation mechanisms, a high rate of SR generation, the increasing rate of guanine oxidation and activity of matrix metalloproteinases in AT adjacent to tumors are important factors in tumor progression. These indicators may have the prognostic value for SC and CC patients.

PROGNOSTIC SIGNIFICANCE OF EXPRESSION OF TUMOR SUPPRESSOR microRNA 122, 133a, AND 200b IN BREAST CANCER PATIENTS

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In recent years it is evidenced the usefulness of the new epigenetic markers, especially miRNAs for prognosis of a course of cancer disease. It was shown that the development of tumors of various origins, including breast cancer (BC) is accompanied by a reduction in tumor suppressor miRNAs targeting oncogenes which are involved in regulation of cell division, invasion, apoptosis, proliferation, and metastasizing. We wanted to determine the relationship between expression of miR-122, -133a and -200b in tumor cells and the clinico-pathological parameters of BC to assess their prognostic significance. The study involved 145 patients with BC at the stage I–III. Expression of miR-122, -133a, and -200b in tumor cells was determined by the real time RT-PCR. As a control, 14 samples of visually intact breast tissue were used. We have found that expression of miR-122, -133a, and -200b was diminished 5.2 ± 0.67, 2.8 ± 0.34, and 4.1 ± 0.29 folds, respectively, in BC samples in comparison with normal breast tissue. There is a correlation between expression levels of all of the studied miRNAs and the basic clinical and pathological characteristics of BC. In particular, we found the inverse correlation of miR-200b, -133, and -122 with the presence of regional metastases in lymph nodes ($r = -0.46$, $r = -0.38$, $r = -0.4$, respectively; $p < 0.05$). The lowest levels of miR-122 (0.040 ± 0.006 relative units) and miR-200b (0.050 ± 0.007 relative units) were detected in the cells of the triple-negative (ER–, PR–, HER2–) BC, compared with other molecular subtypes. Also we have found that the reduced expression of miR-133a (<3 relative units) in tumor tissue is associated with the high risk of recurrence of BC ($r = 0.49$). ***Conclusions***: The correlation of expression of tumor suppressor miR-122, -133a, and -200b with clinical and pathological characteristics of BC, such as metastases, basal molecular subtype and the disease-free survival make it possible to use these microRNAs as the additional prognostic markers of BC progress.

CHANGES IN SYSTEM OF CELL REGULATING REDOX- AND GLYCOLYTIC FACTORS IN WALKER-256 CARCINOSARCOMA TISSUES WITH DIFFERENT SENSITIVITY TO DOXORUBICIN

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Zn-dependent matrix metalloproteinases (MMP)-2 and -9 play an important role in tumor progression, especially in the invasion, metastasizing and angiogenesis. We wanted to investigate the relationship between glycolytic and redox indicators, i.e. the number of "free iron" (FI), the generation of superoxide radical anion (SRA), the ratio of Cu/Zn, and levels of lactate dehydrogenase (LDH), ferritin (FR), and also the MMP-2 and -9 activity in tumor tissue (TT) of animals with different sensitivity to doxorubicin. Studies were conducted *in vivo* on two groups of animals with carcinosarcoma Walker-256, original and with induced resistance to doxorubicin. In two groups of animals we have found the oppositely directed changes in glycolytic and redox indicators. In sensitive to doxorubicin animals there were increased levels of FI and

Cu/Zn 1.2 fold, SRA — 3.1 fold, LDH — 1.4 fold. Other direction of changes was observed in resistant to doxorubicin animals: decrease of FI 1.6 fold, Cu/Zn and SRA — 1.2 fold, LDH — 1.3 fold. Regardless of the sensitivity of cells to doxorubicin, the higher levels of FR were detected in carcinosarcomas. Parallel to that we have observed multidirectional changes in the activity of MMP-2 and -9. In TT, sensitive to doxorubicin their activity increased 1.4 and 1.2 fold, respectively, while in the resistant TT the 1.4 and 1.6 fold decrease was detected. ***Conclusions*:** Changes in glycolytic and redox indicators, the ratio of Cu/Zn, detected after doxorubicin treatment in tumor tissues with different sensitivity to it, are associated with activation or inhibition of MMP-2 and -9. This may be used to correct the metastatic and angiogenic activity of the transformed cells to increase their sensitivity to the action of anticancer drugs.

CYTOTOXIC EFFECTS OF EXOGENOUS LACTOFERRIN ON BREAST CANCER CELLS WITH DIFFERENT DEGREE OF MALIGNANCY *IN VITRO*

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Experimental studies have shown that the highest activity as antioxidants, compared with synthetic analogs, has a natural antioxidant protein lactoferrin (LF). We wanted to investigate the impact of exogenous LF on breast cancer cell lines of different degrees of malignancy *in vitro*. To do so, we have chosen cells with a low (MCF-7, T47D) and the high degree of malignancy (MDA-MB-468, MDA-MB-231). LF was added in a dose at the IC_{30} (100 mg/ml) for 48 h. It was observed that LF induced apoptosis. The cells of the high degree of malignancy, MDA-MB-468 and MDA-MB-231 showed the highest number of the dead cells (43.2 ± 2.5 and 41.4 ± 2.1%, respectively). This was accompanied by downregulation of Bcl-2 by 19.5%, and increased expression of pro-apoptotic protein Bax by 13.5%. The DNA in comet tail for MDA-MB-468 and MDA-MB-231 cells showed a significant increase too, at 3.9 ± 0.5 and 3.8 ± 1.2% fold, respectively ($p < 0.05$). The observed cytotoxic effects of LF were accompanied by the cell cycle arrest in G2/M phase. ***Conclusions*:** The peculiarities of cytotoxic and genotoxic action of exogenous LF indicate possibility to use it to modify the phenotype of breast cancer cells of the high degree of malignancy.

PATTERN OF EXPRESSION OF CANCER STEM CELLS MARKERS, CD44 AND CD24, IN MALIGNANT PROSTATE TUMORS

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In recent years, there is growing body of evidences that a distinct subpopulation of tumor cells, i.e. cancer stem cells (CSC, cancer initiating cells) exists. The presence of these cells is a determining factor for the growth and progression of tumors, their metastatic activity, and sensitivity to chemo- and radiotherapy. We aimed to investigate the expression pattern of the CSC markers, such as CD44 and CD24 in prostate malignant tumors and find a relationship between their expression and pathological characteristics of patients. The study involved 86 patients with prostate cancer at the stages II–III. The presence of the CD24 and CD44 antigens in tumor tissue section was found in 52.3 and 75.6% of the total number of tumor samples, respectively. Number of tumors that showed expression pattern as $CD44^+/CD24^{-/low}$ in CSC was 32.5%. The largest number of $CD44^+$ and $CD44^+/CD24^{-/low}$ tumors was identified in patients at the stage III (77 and 53%, respectively) compared with patients with prostate cancer of the stage II (65 and 28%, respectively). We also found that high levels of prostate specific antigen (PSA, > 15 ng/ml) that was detected in the serum of prostate cancer patients was associated with increased number of $CD44^+$ and $CD44^+/CD24^{-/low}$ cells. We have found that patients with a CSC phenotype as $CD24^-$ and $CD44^+/CD24^{-/low}$ showed a higher (3.0 and 3.3 fold) incidence of relapse compared with patients with other tumor cell phenotype. ***Conclusions*:** We found a correlation between patterns of CD24 and CD44 expression and clinical and pathological features of prostate cancer. This suggests that expression of CSC markers may be used as additional criteria for prognosis of the course of disease.

CLINICAL SIGNIFICANCE OF THE ISOLATED TUMOR CELL DETECTION IN THE PERITONEAL LAVAGE SOLUTION IN PATIENTS WITH RESECTABLE PANCREATIC DUCTAL ADENOCARCINOMA

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Clinical role of the isolated tumor cells (ITC) in peritoneal lavage solution of pancreatic cancer (PC) patients is disputable. The aim of this study was to evaluate the influence of ITC in peritoneal lavage of PC patients after curative resection on overall survival (OS) recurrence free survival (RFS) and investigate the role

of surgery in peritoneal dissemination of PC. 22 resected patients with PC were consequently involved into the study. PC was proved histologically in all cases. All patients underwent adjuvant chemotherapy. Median age was 63 (45–74) years. Peritoneal lavage was aspirated from 3 areas intraoperatively. Aspiration was performed at laparotomy and at the end of surgery. Paraffin blocks were made of the aspirates sediment. Cytological samples were investigated histologically and immunocytochemically (ICC). CEA monoclonal antibodies (MAB) (clone II-1, DAKO, Danmark) and CA 19-9 MAB assays (clone C241:5:1:4, Diagnostic Biosystems, USA) were applied. ITCs were registered in none of 22 patients at laparotomy. In 2 patients ITCs were identified by ICC. No influence on patient prognosis was seen when analyzing OS and RFS. Median RFS was 12 months. ***Conclusions*:** At present, ICC method and cytological examination can't be used as an adequate tool in evaluation of intraoperative peritoneal dissemination of PC or in prognosis of resectable patients with PC.

WHAT IS THE MOST EFFICIENT IMMUNOTHERAPY TO EPSTEIN — BARR VIRUS (EBV) INFECTION?

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Epstein — Barr virus (EBV) is the most common pathogenic virus in the human population with a prevalence globally > 90%. As a herpesvirus it remains latent after primary infection throughout life. Every year EBV is involved in the pathogenesis of some 200,000 new cancers, affecting the immune system, nasopharynx or stomach. The viral carcinogenesis depends on cofactors such as genetic, co-infection or immunologic (e.g. immunosuppression). In healthy people primary infection often results in infectious mononucleosis (IM), a self-limiting lymphoproliferative disease, particularly if it is delayed to adolescence. We study EBV-related complications and cancers in HIV-infected patients, transplant patients and in the Chinese population with high risk of nasopharyngeal cancer (NPC; 1–5). In this lecture the most appropriate type of immunotherapy against EBV-infection and its complications will be discussed. Restoration of host cellular immunity and/or adoptive T-cell therapy has been successfully employed to block post-transplant lymphoproliferative disease (PTLD) and lymphomas in immunosuppressed patients (3–5). NPC is usually treated with radio- and chemotherapy. In the future NPC should be subject to immunotherapy trying checkpoint inhibitors. ***Conclusions*:** There is an efficient vaccine to EBV directed against its main receptor ligand gp 350. However it is hard to define a target population for cost-effective preventive vaccination, given the wide dissemination of the virus, the nature of primary infection and of the host-virus interaction. On the other hand, immune based therapies will not likely gain increased attention.

THE GENETIC DETERMINANT OF ENDOMETRIAL CANCER SUSCEPTIBILITY

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Earlier, we have identified differences in the contribution of genetic factors to cancer of the female reproductive system (FRS): genetic component in the development of endometrial cancer (EC) is 61.0%, breast — 56.0%, ovary — 67.0%, and cervix — 3.0%. However, the specific clinical indicators relative to their importance for the emergence of tumors of various origins, including EC, has not been evaluated. We aimed to determine the most informative indicators associated with the occurrence of EC. The clinical and genealogical analysis of 218 patients with EC was performed. The special software packages of genetics and mathematical analysis were used. By the analysis of the pedigrees of patients with EC we have found that in the families of 144 (65.5%) patients no aggregation of cancer pathology was observed. Noteworthy, for 77 (34.5%) patients the FRS, colon, stomach, and malignant neoplasms of other origin were found among relatives of the first and the second degrees. In EC patients with aggregation of FRS in genealogy, the informative significance were attributed to: age of occurrence of cancer before 45 years (I = 0.50) and in the ages of 55–65 years (I = 0.50); clinical parameters, such as the number of abortions — more than 4 (I = 0.75), and late onset of menarche — 15 years and over (I = 0.58). It should be noted that in patients with EC with a family history of cancer there is hidden chromosomal instability (inductor bleomycin), informativity of which was 0.86. ***Conclusions*:** our research revealed a set of the most informative features that determine the risk for the occurrence of EC.

EXPRESSION OF CD150 ISOFORMS IN NORMAL AND MALIGNANT B CELLS AT THE DIFFERENT STAGE OF MATURATION

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CD150 (IPO3/SLAMF1) is multifunctional type I transmembrane glycoprotein that belongs to SLAM family within the immunoglobulin superfamily of surface receptors. Within B-cell lineage cell surface receptor CD150/SLAMF1 is broadly expressed starting from pre-B cells with upregulation toward plasma cells. However, expression of CD150 is rather limited on the surface of malignant B cells with the block of differentiation at the different stages of maturation. The existence of alternatively spliced CD150 isoforms with different signaling properties indicates that they may mediate multiple functions by differential expression in lymphoid population at different stage of cell maturation. The aim of our work was to explore

CD150 expression both on protein and mRNA levels with the emphasis on CD150 isoforms in malignant B-cell lines at the different stages of maturation in comparison with their normal B cell counterparts. Protein CD150 expression was accessed by western blot analysis and the expression level of CD150 isoforms was evaluated using qRT-PCR.

Despite the similar CD150 expression levels both, at the mRNA and protein levels in normal B-cell subsets and B-lymphoblastoid cell lines, malignant B-cell lines demonstrated substantial heterogeneity in CD150 expression. The CD150 protein was detected only in Hodgkin's lymphoma cells, Burkitt's lymphoma cell lines BJAB and Raji, and also pre-B cell line BLIN-1. At the same time, total CD150 and mCD150 mRNA was detected in all studied cell lines, except pre-B cell line REH. The minor sCD150 isoform was found only in Hodgkin's lymphoma cell lines and Burkitt's lymphoma cell line Raji. The nCD150 mRNA was revealed in all tested cell lines with the exception of pre-B ALL cell line REH and BL cell line Daudi. Similarly to normal B-cell subsets, in malignant B-cell lines expression level of nCD150 was tenfold lower, compared to the expression of mCD150 isoform. ***Conclusions*:** Malignant B-cell lines at the different stages of maturation resemble their normal counterparts partially, concerning CD150 expression pattern. CD150 isoforms are differentially expressed in normal and malignant B cells with predominant expression of mCD150 isoform.

BODY MASS INDEX AND TUMOR-ASSOCIATED MACROPHAGES IN PATIENTS WITH GASTRIC CANCER: ASSOCIATION WITH THE COURSE OF DISEASE

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Obesity increases the risk of cancers and worsens the disease. Infiltration by macrophages is a common feature in inflammation and obesity. It is shown that adipose tissue that surrounds the tumor or metastases in the lymph nodes serves as the energy source for tumor growth and depots macrophage, stimulating angiogenesis and the pro-tumor functions as tumor-associated macrophages (TAM). We aimed to determine the relationship between body mass index (BMI), number of TAM and overall survival (OS) of patients with gastric cancer (GC). The 128 GC were studied, grouped by BMI (< 25 kg/m^2 — normal body weight, ⩾ 25 kg/m^2 — overweight and obesity). Immunohistochemistry was used to determine TAM (CD68+ cells). Patients with a BMI ⩾ 25 kg/m^2, regardless of age, showed a significant greater number of TAM than people of normal weight. In patients aged < 60 years, TAM positive correlation with BMI is more pronounced ($r = 0.4$, $p < 0.05$). Patients whose tumors are characterized by a lot of TAMs have lower life expectancy and the higher risk of adverse disease, than patients with low TAM (log-rank test: $p < 0.01$, HR = 2.5). ***Conclusions*:** In patients with GC overweight and obesity there is more intense infiltration of TAM in tumor tissue than in patients with normal weight. Number of TAMs is associated with BMI patients and can be used for prognosis of GC.

ADAPTOR PROTEIN Ruk/CIN85 PROMOTES LUNG METASTASIS OF 4T1 MURINE BREAST ADENOCARCINOMA CELLS

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Adaptor proteins consist of several binding domains and motives and usually do not have any catalytic activities. Through interaction with their binding partners, adaptors play crucial role in organization, structuring and regulation of multimolecular complexes, involved in the control of cell responses. Adaptor protein Ruk/CIN85 is involved in motility, invasion, and adhesion of murine 4T1 breast adenocarcinoma cells. The aim of present study was to investigate the effect of Ruk/CIN85 on 4T1 cells metastasis *in vivo*. In order to explore Ruk/CIN85 impact on metastasis, we generated 4T1 sublines with stable Ruk/CIN85 overexpression. $5 \cdot 10^5$ cells were injected into the tail vein of 8 weeks old Balb/c female mice. After 24 h the mice were sacrificed and lungs were excised. Amount of migrated cells was evaluated with lung seeding assay, and lung permeability assay was performed using Evans blue dye. For experimental metastasis assay we injected $5 \cdot 10^5$ cells into the tail vein and after 14 days collected lungs for metastasis assessment. Statistical analysis was performed, using Mann — Whitney U-test. We have found that the mice injected with Ruk/CIN85-overexpressing 4T1 cells are characterized by increased amount of cells, which invaded into lungs, and higher lung vessels endothelium permeability in comparison to control mice. Experimental metastasis assay showed higher number of lung metastasis as well as lung to body mass ratio in group injected with Ruk/CIN85-overexpressing cells when compared to control. ***Conclusions*:** Taken together, these data indicate higher metastatic potential of 4T1 cells with Ruk/CIN85 overexpression.

THE STUDY OF THE NUMBER OF TUMOR-INFILTRATING FOXP3 POSITIVE LYMPHOCYTES IN ENDOMETRIAL CARCINOMAS IN DEPENDENCE ON THE MISMATCH REPAIR STATUS IN PATIENTS WITH A FAMILY HISTORY OF CANCER

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Endometrial cancer (EC) can occur in a patient from a family with the history of a number of hereditary cancer syndromes, the most common of them is Lynch syndrome. The Lynch syndrome is characterized

by germline mutations in mismatch DNA repair genes (MMR). It is known that disruption of MMR-system leads to a large number of immunogenic proteins, resulting in a high lymphocytic infiltration in such tumors. We aimed to estimate the number of FOXP3$^+$ intra-tumor lymphocytes in ECs in dependence on a status of MMR system in patients with a family history of cancer. The surgical samples of 49 patients of the stage I–II of EC were studied. Patients did not receive any treatment before surgery. By the clinical and genealogical analysis of pedigrees of EC patients we found that 26.5% of probands had cancer family history. Most families have accumulated tumors of the female reproductive system and the gastrointestinal tract. We have shown that the number of EC patients with MMR-deficient tumors was significantly greater in patients with family cancer history (61.5%), compared with the group of patients without aggregation of cancers in families (27.8%). Microsatellite instability resulting from the disruption of MMR system was found in 10.7% of EC patients, including one patient with a family of aggregation Lynch-associated tumors. We have also found that in EC patients with the family history the high number of FOXP3$^+$ lymphocytes were detected in 40% of MMR-deficient tumors; while among cancers with normal MMR infiltration of FOXP3$^+$ lymphocytes was significantly higher, up to 66.7%. Most MMR positive carcinomas with a high number of FOXP3$^+$ intra-tumor lymphocytes were characterized by a low degree of differentiation and the deep tumor invasion into the myometrium. ***Conclusions*:** We have found that the infiltration of FOXP3$^+$ lymphocytes in EC correlates with tumor MMR status in patients with a family history, and also is associated with tumor progression.

CLINICAL BENEFIT OF PERSONALISED DENDRITIC CELL BASED VACCINE THERAPY ACCORDING TO THE PD-L1 TUMOR EXPRESSION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Modern antitumor vaccination therapy is one of the most promising strategies in lung cancer biotherapy today. Earlier, promising results from phase III trial of dendritic cell based vaccine immunotherapy for the non-small cell lung cancer (NSCLC) patients at IIB–IIIA stages have been obtained by National Cancer Institute of Ukraine. It is known that the PD-1/PD-L1 pathway plays an important role in blunting immune response to tumor vaccines. The predictive value of PD-L1 tumor expression is controversial. We speculate that DC-vaccine will have more benefit in NSCLC with low/– PD-L1 expression in tumors. Therefore, our main goal is to examine the clinical benefit of personalized DC-vaccine therapy in NSCLC patients, according to PD-L1 expression. The following original design of DC-vaccine was used: autologous DCs of monocytic origin were loaded with mechanically heterogenized microparticles of tumor cells. Maturation state and functional activity of DCs were evaluated by the expression pattern of CD83/86, HLA-DR, and IL-12, and also the p35/p40 mRNA levels. The $4.62 \pm 0.37 \cdot 10^6$ of DCs were injected intravenously in 1–3 courses with 6 months interval. One course consisted of 5 injections with one-month interval. Clinical and immunological monitoring was performed. The PD-L1 expression was detected in primary tumors by real-time RT-PCR. Tumors were categorized into groups, according to high or low PD-L1 expression levels based on cutoff point (AUC = 0.79; $p < 0.03$). Sensitivity and specificity of PD-L1 gene expression as a predictive biomarker for NSCLC patients that received DC-vaccine therapy were 75 and 83%, respectively. Cox proportional hazard regression analysis revealed that PD-L1 expression has no effect on the 5-year overall survival rate for NSCLC patients; it has a significant impact on 5-year event-free survival (EFS). The lower expression of PD-L1 correlates with a significant increase in EFS ($p = 0.026$). The 2-year EFS rate for patients with low PD-L1 expression was 86% compared to 29% for high expression (F-Cox criterion: F = 4.68; $p = 0.017$). ***Conclusions*:** Expression of PD-L1 in tumors has a predictive role for personalized DC-vaccine therapy. Obtained results might serve as a background for the new therapy schemes, based on PD-1 inhibitors and/or specific immunotherapy.

PROTECTIVE ACTION OF QUERCETIN IN EXPERIMENTAL MODEL OF PULMONARY FIBROSIS: EFFECT ON ANTI-INFLAMMATORY POLARIZATION OF PHAGOCYTES IN ALVEOLAR LAVAGE

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It is known that toxic manifestations of anticancer chemotherapy limit significantly treatment outcomes of cancer patients and worsen their quality of life. One manifestation of side effects of anticancer drugs is the formation of fibrosis of lung and/or heart. One of the main causes and prerequisites of fibrosis is inflammation, when polarization of phagocytes is observed. One of the promising methods to prevent fibrosis is activation of the anti-inflammatory function of phagocytes by bio-flavonoids (characterized by a wide range of pharmacological activities, including antioxidant). We have investigated the ability of quercetin to stimulate polarization of inflammatory phagocytes in alveolar lavage of mice and adjust development of pulmonary fibrosis by bleomycin. The study was conducted on S57Vl/6 mice. 28 days after bleomycin administration the number of infiltrating the lungs phagocytic cells and their phagocytic activity and production of reactive oxygen species (ROS) were determined. The introduction of quercetin at doses of 0.45 and 4.5 g/kg resulted in reduction of infiltrating

and total number of lung phagocytes, compared to the control, up to 38.5% ($p < 0.05$) and 42.3% ($p < 0.05$), respectively. Integral phagocytic activity of immune cells in the lung lavage of mice upon quercetin treatment increased significantly, due to activation of neutrophils absorbance on 65.1 and 90.3%, respectively. ***Conclusions*:** Quercetin shows the protective effect upon the formation of bleomycin-induced lung fibrosis, when administered at a dose of 4.5 g/kg. This is evidenced by a decrease in lung infiltration by phagocytic cells, their polarization towards the anti-inflammatory effects, and by reduced content of hydroxyproline in the lungs of experimental animals.

CYTOGENETIC MARKERS OF INITIATION AND PROGRESSION OF CANCEROGENESIS IN DIFFERENT STRAINS OF MICE

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Multistage carcinogenesis is one of the main reasons why it is difficult to identify the key events. We wanted to expose a link between chromosomal instability and increased sensitivity for tumor promotion in laboratory mice and to identify the most informative cytogenetic characteristics, useful for assessing the stage of spontaneous and induced cancer. We studied the mutational spectra in bone marrow (BM) cells of mice BALB/c, C57BL/6, DBA, and ICR at the control conditions and upon induction of tumors (melanoma B-16, breast adenocarcinoma Ca755, spontaneous carcinogenesis in ISR mice). We have found that in the control BM cells in young mice of high tumor incidence — BALB/c, DBA, and ICR — the cytogenetic instability was manifested long before tumor development. With age, ICR mice showed the increasing levels of aneuploidy in BM cells, and also the high number of cells with chromosomal aberrations, asynchronous splitting of centromeric regions of chromosomes, chromosome associations by type of Robertson translocations, the high frequency of lymphocytes with micronuclei, and also mitotic, apoptotic cells, nuclear abnormalities, pathological mitosis. ***Conclusions***: Reduction of chromosomal stability is closely associated with tumor stage; the increased number of mitotic cells contributes to tumor progression.

INFLUENCE OF IFN-beta GENE TRANSDUCTION ON SENSITIVITY OF TUMOR CELLS TO CHEMOTHERAPY

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One of the main treatments for cancer patients is the combined chemotherapy, which may include cytokines or the expression vectors, bearing genes, encoding cytokines. Also, it is important to determine the effectiveness of a drug combination to identify synergistic or antagonistic nature of their interaction. The aim of our work was to study the sensitivity of tumor cells that were transduced by a recombinant baculovirus, bearing the IFN-beta (rBV/IFN) to the chemotherapy. Melanoma B16 cells (MM4) and Lewis lung carcinoma cells (LL) were chosen for transduction. We have found that transduction of MM4 cells by rBV/IFN and led to a significant increase in sensitivity of melanoma cells to dacarbazine (an anti-metabolite). Thus, IC_{50} of dacarbazine to control MM4 cells was 100 mg/ml, and for transduced MM4 /rBV/IFN cells — only 10 mg/ml. Unlike melanoma cells, transduction of the LL cells by rBV/IFN led to a significant increase in their sensitivity to cisplatin, docetaxel and etoposide. Thus, for the control cells IC_{50} of cisplatin was 0.5 mg/ml, and cells for transduced cells — 0.25 mg/ml; IC_{50} of docetaxel for the control cells amounted to 5 ng/ml, and for transduced cells — 1.5 ng/ml. Integrated action of etoposide on transduced LL/rBV/IFN increased also their sensitivity to the drug, two-folds. ***Conclusions*:** Our data suggest that the effectiveness of combination gene- and chemotherapy can be personalized. It will depend largely on several conditions, including the tumor type, sensitivity of tumor cells to IFN and chemotherapy, the cell cycle phase after transduction, and also other factors.

CHARACTERIZATION OF METHYLATED VIM, TMEFF2 AND GDF15 AS A POTENTIAL SET OF EPIGENETIC MARKERS FOR BLADDER CANCER

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Bladder cancer is one of the most common cancers in Ukraine and in the world and a problem of its early diagnosis is still unresolved. The use of epigenetic markers, such as methylation of tumor suppressor genes, is a promising tool to assess the risks of the disease and its early detection. The aim of this study was to evaluate the potential of combination of tumor suppressor genes GDF15, TMEFF2, and VIM as diagnostic and differentiation markers for bladder cancer. The urine from patients with bladder (sample size is n = 42), renal (n = 13) and prostate cancers (n = 13) were collected, as well as from 5 healthy individuals as negative controls. DNA was precipitated by CTAB and isolated, using the standard phenol/chloroform/isopropanol method. Methylation of VIM, TMEFF2,

and GDF15 promoters was detected by RealTime MSP PCR after the DNA bisulfite conversion. We found the presence of hypermethylation of at least one of 3 studied genes in 69% of bladder cancer samples, while no methylation was detected in samples taken from healthy people. Among renal and prostate cancer samples VIM, TMEFF2 and GDF15 were not methylated in 11 from 13 samples and in 12 from 13 samples, respectively. ***Conclusions:*** Our results are an evidence of possibility to use promoter methylation of GDF15, TMEFF2, and VIM to distinguish between bladder cancer and other uro-oncological diseases. The number of patients and healthy individuals should be increased, to prove our preliminary results.

CURRENT METHODS TO STUDY THE PROTEOME OF BREAST CANCER

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According to epidemiological data, breast cancer (BC) — one of the most common cancers of the female population in the world. According to the National Cancer Registry (2014) 13 641 new cases of BC and 5874 new cases of deaths due to this disease were registered in Ukraine. In the last decade, much attention is paid to the proteomic research as an integral part of the functional genomics of tumors. The most essential results of studies on the diagnostic features, action of therapeutic drugs, identification of predictive factors and tumor biomarkers are and will be introduced in diagnostics and treatment in clinical practice. We reviewed the literature for the optimal technical solutions to perform the proteome research of breast cancer. Different types of mass spectrometry is used, especially the peptide-oriented Proteomics and methods based on matrix. Using the described methods, the special groups of BC, associated with the mutated BRCA1 and BRCA2 were established, and also the biomarkers for lobular and ductal carcinomas were characterized as well. Protein expression patterns of three receptor negative breast cancers were compared with HER's-2-positive tumors and more than 95% ER negative cases of BC with a poor prognosis were classified. ***Conclusions*:** MS technology plays a key role in the modern achievements of proteomics standards to improve diagnosis, treatment and prognosis.

PROLIFERATION ACTIVITY AND EPITHELIAL-MESENCHYMAL TRANSITION IN HEPATOCELLULAR CARCINOMA: A PILOT STUDY

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Liver cancer is the fifth most frequently diagnosed cancer and the second leading oncologic death cause worldwide. The high mortality from hepatocellular carcinoma (HCC) is mainly attributed to the invasion pattern and intrahepatic and/or extrahepatic metastases, but the exact mechanism remains unclear. Yet, the escape of neoplastic cells from the solid tumor might be due to de-differentiation which occurs by loss of cell-to-cell contacts and the concomitant gain of migratory and invasive abilities. This phenotypical conversion of cells, collectively designated as epithelial-mesenchymal transition (EMT), has been described in different types of carcinoma including HCC. We aimed to evaluate the proliferation activity (by Ki-67) and EMT (by vimentin and E-cadherin) of hepatocellular carcinomas. In a retrospective study, 50 consecutive morphologically confirmed cases of HCC were enrolled. There were 36 (72.0%; 58.2–82.6) males and 14 (28%; 17.4–41.8) females. The mean age was 63.8 ± 9.4 years (residual range 42–82). The expression of Ki-67, vimentin and E-cadherin was detected by immunohistochemistry. The proliferation fraction was scored quantitatively (%) in the neoplastic nuclei. The mean proliferation fraction was 26.1 ± 18.2%. Expression of vimentin and E-cadherin was evaluated semi-quantitatively by intensity (scale, 0–3) and the fraction (%) of positive neoplastic cells. The mean expression of E-cadherin was 1.5 ± 1.0, comparable with the moderate intensity of peritumoral benign hepatocytes (1.7 ± 1.0) but being less than in reactive bile ducts (2.7 ± 0.4). HCCs were mostly negative (85.0% of cases) for vimentin. The hepatocytes were invariably negative while reactive bile ducts showed higher expression. Currently, there is a considerable body of literature indicating that hepatocellular EMT is a crucial event in HCC progression. However, expression of EMT marker vimentin was a rare event in the present group. Our data match with other studies, suggesting that HCC is a discohesive malignancy with low E-cadherin expression. Reduced E-cadherin expression correlates with lower overall survival for HCC patients, metastasis and vascular invasion. ***Conclusions*:** HCC is tumor with low proliferative activity indirectly indicates low efficacy of chemotherapy.

IS THE MRPS18-2 ONCOPROTEIN A MASTER REGULATOR OF CELL DIFFERENTIATION?

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We have found that mitochondrial ribosomal protein S18-2 (MRPS18-2) is involved in regulation of the RB-dependent pathway. It binds to both, hypo- and hyperphosphorylated RB protein. The binding between RB and S18-2 proteins is promoted when cytoplasmic S18-2 is targeted to the nucleus, and this disrupts the association of E2F1 with RB, as indicated by the increased level of free E2F1 in the nucleus. This presumably lifts the RB dependent block to S-phase entry in the cell cycle. We have also found that over-

expression of the human S18-2 immortalized primary rat embryonic fibroblasts and they showed properties of embryonic stem cells. Terminally differentiated skin fibroblasts were transformed upon S18-2 overexpression. Moreover, S18-2 increased in endometrial cancers compared with the normal endometrium and hyperplasia, based on a study of 42 patient biopsies. Elevated expression of S18-2 in stem and tumor cells (our findings and analysis of published microarray data) raises the question of whether this protein co-operates with the RB protein in differentiation and cancerogenesis. In the present work we aimed to characterize the pathways of cell fate regulation with the involvement of S18-2 and RB proteins. We showed that S18-2 protein, together with RB, plays a crucial role in cell dedifferentiation. We have found that overexpression of S18-2 and RB is needed for maintenance of cell stemness. Such cells can differentiate into various cell lineages under certain conditions. We have shown that S18-2 could enhance the telomerase activity. Overexpression of S18-2 induced chromosomal instability in the transfected cells. ***Conclusions***: The 18-2 is a novel oncoprotein that also is involved in control on cell stemness.

ESR1 GENE POLYMORPHISMS IN PATIENTS WITH BENIGN DISORDERS OF THE FEMALE REPRODUCTIVE SYSTEM, IN DEPENDENCE ON CANCER HISTORY IN FAMILY

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It is not known how the single nucleotide polymorphism (SNP) of the estrogen receptor gene (*ESR1*) is associated with benign disorders of the female reproductive system (FRS). Such studies are badly needed for women with oncogenetic syndromes, when tumors of FRS are inherited. We wanted to interview patients with benign pathology of FRS from families with a family cancer syndrome and also to determine the frequency of polymorphisms, such as T397C and A351G in ESR1. We analyzed the questionnaires of the medical genetic counseling and isolated genomic DNA of peripheral blood of 120 women (aged 23 to 83 years), including 65 with benign pathology of FRS (uterine, ovarian, breast). In the families there was the cancer syndrome (Lynch II). The 55 healthy women aged 19–78 years were analyzed similarly; there was no aggregation of malignant tumor diseases in pedigree. The results of a comprehensive clinical and clinico-genealogical research have shown that in families of 65 probands with benign pathology of FRS, there were malignancies mostly of FRS (cancer of ovary, breast, endometrium), and also of gastrointestinal tract (colon and gastric cancer) and others, that corresponds to the Lynch syndrome type II. In families of probands with benign disorders of the breast, uterus, and ovary from families were cancer of RFS were detected, we have found an aggregation of malignant tumors of the type of family cancer syndrome and also significant changes in the frequency of T397C polymorphisms, while no changes in the frequency of A351G polymorphism. Statistical calculations showed that in terms of odds ratio the risk of benign pathology of FRS is the largest by bi-allelic SNP of the *ESR1* gene (T397C) and this risk is 5.2 at 95% confidence interval (1.42–19.06). ***Conclusions***: Our results can be used to create the genetic risk groups to develop benign tumors in women from families with a family cancer syndrome.

RELATIONSHIP BETWEEN LEVELS OF COPPER- AND IRON-CONTAINING PROTEINS AND CLINICAL AND PATHOLOGICAL FEATURES OF BREAST CANCER

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Breast cancer (BC) is one of the most common forms of cancer diseases in many countries. The problem is not only that the BC in often asymptomatic at the early stages, but also in the absence of reliable markers for predicting its course. According to the literature, copper- and iron-containing proteins might be considered as potential markers of prognosis of malignant progression and also the promising target for anticancer therapy. We have examined the relationship of ceruloplasmin (CP) and transferrin (TF) in serum and tumor tissue of BC patients with their clinical and pathological characteristics, including molecular subtype. We studied the 276 BC patients at the stage I–III and of age 23–89 years (average age was 55.4 ± 7.3). In 58.7% of patients with BC the CP level in serum was significantly higher than in relatively healthy women, and the TF level was below the performance standards in 62.3% of patients. Also we have found that CP levels correlate directly with the stage of disease and the development of metastases in regional lymph nodes. Noteworthy, the TF levels in the blood serum were dependent on the histological tumor types. We found that high TF levels observed frequently in the serum of patients with stage I BC ($r = -0.28$) and luminal subtype A tumors ($r = 0.45$). The lowest level of TF was observed in patients with luminal subtype B tumors and in basal cancers ($r = 0.35$). Increased expression of TF was more characteristic for BC patients with a low degree of differentiation ($r = -0.39$), this correlated with the presence of metastatic lymph nodes ($r = 0.48$). The largest number of CP positive tumors (80%) was observed in the group of Her2/neu positive cancers. ***Conclusions***: Our data on the relationship between levels of CP and TF and the clinical and pathological parameters and molecular subtypes is a promising line for the further research, and may be used as additional prognostic markers of the course of tumor disease.

ABILITY OF QUERCETIN TO CORRECT THE PARANEOPLASTIC HEMATOLOGICAL SYNDROME

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It is known that a malignant tumor is able to cause the development of paraneoplastic syndrome (PNS). Among the various manifestations of PNS a special place is taken by hematology. The use of drugs for therapeutic correction of PNS is limited due to their toxicity and the risk of stimulating cancer. We wanted to investigate the ability of quercetin to counteract the hematologic manifestations of PNS, induced by tumor growth. The C57BL mice with the Lewis lung carcinoma (LLC/R9) were used as a model. Treatment with quercetin started the day after inoculation of LLC/R9 cells into mice. Growth of LLC/R9 cells in mice was accompanied by pronounced signs of tumor-associated thrombocytopenia and anemia. Quercetin had no influence on red blood cells. However, the bone marrow cells were increased in number. Quercetin treatment resulted in increase of the number of platelets up to 52% ($p < 0.05$), particularly when a dose of 0.45 g/kg was used, compared to the control animals. Moreover, we have found increase in the total number of myelokaryocytes (1.7 fold, $p < 0.05$) and megakaryocytes (2.6 fold, $p < 0.05$). Noteworthy, the fraction of oxyphilic megakaryocytes was larger. Quercetin treatment has not stimulated tumor growth or metastasizing. ***Conclusions*:** The fact that quercetin can diminish the tumor-associated thrombocytopenia without stimulating tumor growth and metastasizing, makes it a putative drug for the support of therapy for cancer patients.

PROSTATE TUMORS CHARACTERIZATION BY NotI-MICROARRAYS

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Prostate cancer is the second most common cancer type among men worldwide. Most of malignant prostate tumors are dormant and don't need any surgery. Some percent of them would progress into aggressive metastatic type. Mechanisms of this transition are still being elucidated. A number of regions in the short arm of human chromosome 3 were found to be frequently deleted or methylated in cancer genomes. We aimed to characterize different types of prostate tumors by chromosome 3 specific NotI-microarray to find the changes and putative biomarkers. NotI-microarray microarray with 180 genomic loci (clones) associated with chromosome 3 was used to detect genetic and epigenetic changes in 15 prostate adenomas and 14 prostate carcinomas. Pool from 4 prostate inflammation samples was used as a reference. NotI-microarray results were verified for selected genes (*FGF12, GATA2, LMCD1*, and *TESSP2* as control) by bisulfite sequencing. Alterations in 158 NotI-clones (genes/loci) were found. There were observed 1624 cases of deletion/methylation and 24 cases of amplification/demethylation. Genes with high percent of deletion/methylation (> 30%) could be divided into three groups: 1) previously not shown to be associated with carcinogenesis; 2) known to be involved in non-prostate cancer; 3) previously shown to be associated with prostate cancer. Some of these genes have significant differences in deletion/methylation frequency in different prostate tumors: 1) high Gleason score carcinoma *vs* adenomas and low, medium Gleason score carcinomas — 9 genes: *LOC440944/SETD5*, *OSBPL10/ZNF860*, *CLCN2*, *FAM19A4*, *PRSS42/MYL3*, *VHL*, *BBX*, *LMCD1*, *CMTM6* ($p < 0.001$); 2) prostate adenoma *vs* carcinoma – 6 genes: *CAND2*, *GATA2*, *FAM19A4*, *KY*, *ALDH1L1*, *MAP4* ($p < 0.05$). Methylation of NotI-site was found in 40–80% of clones for *FGF12* gene, 30–70% — for *GATA2* and *LMCD1* genes, and <10% — for *TESSP2* gene. Bisulfite sequencing proved NotI-microarray results. ***Conclusions*:** Most common changes of chromosome 3 in prostate tumors are deletion and/or methylation. We found 9 genes with significant differences in deletion/methylation frequencies between carcinomas of high Gleason score compared with adenomas and low/medium Gleason score carcinomas; and also 6 genes when prostate adenomas were compared with all carcinomas. NotI-microarray is a powerful tool for screening of genetic and epigenetic alterations in prostate cancer.

DEPENDENCE OF EFFICIENCY OF ANTICLASTOGENIC ACTIVITY OF SODIUM HUMATE IN RADIATION-INDUCED MUTAGENESIS IN PATIENTS WITH THYROID CANCER

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Determining the molecular basis of antimutagenes and anticlatogenes is important not only to prevent, but also to inhibitcarcinogenesis at the different stages. The *XPD* and *XRCC1* genes are important regulators of DNA repair systems. Upon ionizing radiation and action of alkylating agents polymorphisms, such as Lys751Gln in *XPD* and Arg399Gln in *XRCC1* are markers for the patient survival after chemotherapy and also of sensitivity to a range of cytotoxic drugs. We wanted to evaluate anticlastogenic efficiency of the sodium humate (10 mg/ml) after γ-irradiation (^{137}Cs) at a dose of 1 Gy in the culture of peripheral blood lymphocytes of patients with thyroid cancer, depending on polymorphisms Lys751Gln in *XPD* and Arg399Gln in *XRCC1*. In all studied cases, when ho-

mozygous polymorphism was observed for any of the abovementioned genes, the antimutagenic effect of sodium humate was absent or reduced significantly. However, the risk of antimutagenic reduction was not statistically significant, due to the low specificity (0.33) of the marker at simultaneous high sensitivity (1.00). This suggests that polymorphisms Lys751Gln in *XPD* and Arg399Gln in *XRCC1* are not the specific markers of radioprotective efficiency of antimutagenes. Reduction of antimutagenic effectiveness of sodium humate in lymphocytes culture in patients with thyroid cancer may be due to inhibition of the functional activity of other genes. ***Conclusion:*** Our results are prerequisites for development of new approaches for correcting etiopathogenetical mechanisms that are related to inhibition of protective processes upon cancerogenesis.

CARBOSILANE-VIOLOGEN-PHOSPHORUS DENDRIMERS AS POTENTIAL NANOCARRIERS OF ANTI-APOPTOTIC siRNAs IN CANCER THERAPY

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Apoptosis, namely programmed cell death, have a crucial role in development, as well as in maintenance of the tissues. The uncontrolled propagation and loss of the ability to undergo apoptosis of the normal cells lead to transformation into cancer cells. The major regulators of the apoptotic process are the Bcl-2 family proteins. Among the group of the apoptosis inhibitors are Bcl-2, Bcl-xl, and Mcl-2. One of the mechanisms of the suppression of synthesis of anti-apoptotic proteins is a process of selective gene silencing by small interference RNAs (siRNAs). This study examines a perspective of using of two generation of carbosilane-viologen-phosphorus dendrimers as effective and safe carriers of anti-apoptotic siRNAs (siBcl-xL, siBcl-2, siMcl-1) for cancer therapy. The complexes were characterized, using fluorescence quenching, circular dichroism and gel electrophoresis methods. The proliferation of HL-60 (human promyelocytic leukemia) cells in result of interaction with dendriplexes was evaluated by MTT cytotoxicity assay. The results have shown that formation of complexes occurs between all of anticancer siRNAs and carbosilane-viologen-phosphorus dendrimers of both generations. However, the complexes have been formed at different dendrimer/siRNA molar ratio. The cytotoxicity assay has shown that mixture of all 3 siRNAs is more effective than single siRNA. In case of single siRNAs only Bcl-xl complexed with 1st generation dendrimer caused statistically significant decrease in HL-60 cell viability. ***Conclusions*:** Combination of few siRNA on carbosilane-viologen-phosphorus dendrimers is a promising way to induce apoptosis in tumor cells.

EFFECT OF MELANIN, THE AGONIST OF PPARγ RECEPTOR ON THE MUCOUS MEMBRANE OF THE COLON CANCERS

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It is known that one of the factors in the development of colon cancers is long hypergastrinemia that is caused by low levels of HCl in the stomach. The appearance of undifferentiated cells and their proliferation is observed prior malignant transformation of cells of the intestinal mucosa. Therefore, one of the possible ways of preventing the emergence of tumors is the effect on PPARγ receptors as central regulators of cell differentiation, apoptosis and inflammatory reactions. In the recent years, more attention is drawn to natural compounds, among which there is the natural agonist of PPARγ receptor melanin, which has low toxicity. Research conducted at 5-month-old male rats in 3 groups: 1 — control; 2 — omeprazole (14.0 mg/kg) was administered for 28 days; 3 — melanin (0.1 mg/kg) and omeprazole were administered for 28 days. Paraffin sections of the colon (removed and fixed) were stained with anti-p53 antibodies (Dako, Denmark) and Mayer's hematoxylin. We have found that the colon mucosa of rats in the control group corresponded to normal. In the intestinal mucosa of rats in group 2 the deepening intestinal crypts with significant destruction of the epithelial layer was seen. Also, white blood cells were located throughout the mucosal surface, as observed at mucous membrane inflammation. Expression of p53 protein in epitheliocytes was significantly increased compared with the control group. 28-day concomitant administration of omeprazole and melanin prevented the development of changes in the lining of the large intestine of rats in 3rd group: no gland atrophy or undifferentiated cells were observed. Expression of p53 protein in epitheliocytes was weak, which allows to conclude that the significant slowdown dysplastic changes in the mucosa took place. ***Conclusions*:** The introduction of melanin against long hypergastrinemia significantly decreased the carcinogenesis, inhibiting proliferation and degeneration of atypical epithelial cells in the lining of the stomach.

REDOX MECHANISMS OF ANTITUMOR AND ANTIMETASTATIC ACTION OF L-ARGININE HYDROCHLORIDE AND COENZYME Q_{10}

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One of approaches to find the innovative anticancer drugs is the use of compounds — donors of nitric oxide (NO) and molecules that control the speed of electron transport in mitochondria, such as coenzyme Q_{10} (CoQ_{10}). We wanted to investigate the redox-dependent mecha-

nism of antitumor and antimetastatic action of L-arginine hydrochloride (L-Arg) and SoQ_{10} *in vivo*. The study was conducted on the S57VI mice with the introduced Lewis lung carcinoma. The drugs were injected intraperitoneally at high and low doses, i.e. L-Arg (60 and 360 mg/kg body weight of the animal), CoQ_{10} (0.2 and 1.2 mg/kg of the animal), and L-Arg with CoQ_{10} together. The L-Arg in high doses caused inhibition of tumor growth for 48.0 ± 8.0%, metastasizing was decreased, generation of O_2 and NO levels in the tumor rise to the values of 1.23 ± 0.14 and 2.26 ± 0.31 nM/mg·min, the matrix metalloproteinases (MMP)-2 and -9 activity diminished to 3.55 ± 0.80 and 4.8 ± 1.0 relative units, respectively ($p < 0.05$). The L-Arg in low doses stimulated tumor growth, metastasizing and the level of active forms of MMP-2 and -9 were increased to the 8.44 ± 2.7 and 9.8 ± 3.1 relative units, respectively ($p < 0.05$). The CoQ_{10} in high doses reduced the rate of generation of $O_2\cdot$ to 0.44 ± 0.09 nM/mg·min and NO ($p < 0.05$) significantly. Tumor growth was inhibited by 54.0 ± 11.3%. The combined use of L-Arg and CoQ_{10} in high doses caused inhibition of tumor growth by 51.0 ± 7.4%. ***Conclusions*:** The low doses of L-Arg positively modulates the MMPs activity that contributes to the progression of tumors. The use of L-Arg and CoQ_{10} led to such redox state of the tumor, which is characterized by decreased activity of MMP-2 and -9 and, therefore, contributes to inhibition of tumor invasion and metastasis.

ROLE OF HEPCIDIN IN THE FORMATION OF A DEGREE OF MALIGNANCY OF BREAST CANCER

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Despite the development of the new approaches of diagnostics and treatment, treatment outcomes of breast cancer (BC) patients often remain unsatisfactory. The reason for this is genetic and morphological heterogeneity of tumors, which usually is assessed by the degree of malignancy. The lack of clear criteria for determining the degree of malignancy dictates the need to find additional markers associated with an aggressive course of BC. We wanted to monitor the role of hepcidin part in shaping the degree of malignancy. To do so, we used cell lines *in vitro* and clinical materials *ex vivo*. 4 BC cell lines of different degrees of malignancy (T47D, MCF-7, MDA-MB-231, MDA-MB-468) and *ex vivo* clinical material of 110 BC patients of the stages I–II were used. The degree of malignancy of cells was evaluated by the following criteria: receptor status, proliferation activity, adhesive and invasive properties. We have found that hepcidin was expressed differently in the cells lines. The highest expression was observed in cell lines, characterized by a high degree of malignancy, MDA-MB-231 and MDA-MB-468. Expression of hepcidin directly correlated with proliferative indices (r = 0.45) and invasive cell activity (r = 0.51), content of "free" iron (r = 0.43); and inversely correlated with the presence of estrogen (r = −0.39) and progesterone (r = −0.41) receptors. In clinical material hepcidin expression correlated with cell proliferative activity (r = 0.38) and their receptor status (r = −0.33). At the same time hepcidin levels correlated with the stage of the disease (r = 0.27), the development of regional metastases in the lymph nodes (r = 0.41) and a low degree of tumor (r = 0.32). The overall survival of BC patients is significantly worse when hepcidin was expressed, at the background of no estrogen receptor and high proliferative activity of tumor cells. ***Conclusions*:** Our data suggest that hepcidin expression levels in tumor cells may be used as an additional criterion for prediction of clinical course of BC.